ORIGINAL ARTICLE INFECTIOUS DISEASES

Necrotizing soft tissue infections caused by *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* of groups C and G in western Norway

T. Bruun[1,2], B. R. Kittang[1,3], B. J. de Hoog[4], S. Aardal[5], H. K. Flaatten[4,6], N. Langeland[1,2], H. Mylvaganam[7], H. A. Vindenes[8] and S. Skrede[1,2]

1) *Department of Clinical Science, University of Bergen,* 2) *Department of Medicine, Haukeland University Hospital,* 3) *Department of Medicine, Haraldsplass Deaconal Hospital,* 4) *Department of Anaesthesia and Intensive Care, Haukeland University Hospital,* 5) *Department of Research and Development, Haukeland University Hospital,* 6) *Department of Clinical Medicine, University of Bergen,* 7) *Department of Microbiology, Haukeland University Hospital and* 8) *Department of Plastic Surgery, Haukeland University Hospital, Bergen, Norway*

Abstract

Streptococcus pyogenes (group A streptococcus, GAS) is a major cause of necrotizing soft tissue infection (NSTI). On rare occasions, other β-haemolytic streptococci may also cause NSTI, but the significance and nature of these infections has not been thoroughly investigated. In this study, clinical and molecular characteristics of NSTI caused by GAS and β-haemolytic *Streptococcus dysgalactiae* subsp. *equisimilis* of groups C and G (GCS/GGS) in western Norway during 2000–09 are presented. Clinical data were included retrospectively. The bacterial isolates were subsequently *emm* typed and screened for the presence of genes encoding streptococcal superantigens. Seventy cases were identified, corresponding to a mean annual incidence rate of 1.4 per 100 000. Sixty-one of the cases were associated with GAS, whereas GCS/GGS accounted for the remaining nine cases. The in-hospital case fatality rates of GAS and GCS/GGS disease were 11% and 33%, respectively. The GCS/GGS patients were older, had comorbidities more often and had anatomically more superficial disease than the GAS patients. High age and toxic shock syndrome were associated with mortality. The Laboratory Risk Indicator for Necrotizing Fasciitis laboratory score showed high values ($\geq$6) in only 31 of 67 cases. Among the available 42 GAS isolates, the most predominant *emm* types were *emm*1, *emm*3 and *emm*4. The virulence gene profiles were strongly correlated to *emm* type. The number of superantigen genes was low in the four available GCS/GGS isolates. Our findings indicate a high frequency of streptococcal necrotizing fasciitis in our community. GCS/GGS infections contribute to the disease burden, but differ from GAS cases in frequency and predisposing factors.

Keywords: β-haemolytic streptococci, *emm* gene, invasive, necrotizing fasciitis, necrotizing soft tissue infections, *Streptococcus dysgalactiae* subsp. *equisimilis*, *Streptococcus pyogenes*, superantigens
Original Submission: 11 February 2013; **Revised Submission:** 7 May 2013; **Accepted:** 7 May 2013
Editor: F. Allerberger
Article published online: 24 May 2013
Clin Microbiol Infect 2013; **19:** E545–E550
10.1111/1469-0691.12276

Corresponding author: T. Bruun, Department of Clinical Science, University of Bergen, Post box 7804, 5020 Bergen, Norway
E-mail: **trond.bruun@helse-bergen.no**

Introduction

Necrotizing soft tissue infections (NSTI) and the dominating subgroup, necrotizing fasciitis (NF), are rare and serious conditions characterized by rapid spread along tissue planes with development of necrosis [1–3]. NSTI is a heterogeneous group of infections affecting various groups of patients, caused by many microbes, leading to a wide range of clinical manifestations. Data on incidence, predisposing conditions and prognostic factors diverge, probably reflecting differences in inclusion criteria and study populations in various studies [3]. The classification of NSTI has traditionally been based on microbiological findings: type I infections are polymicrobial, whereas type II infections are monomicrobial, and most often caused by *Streptococcus pyogenes* (group A streptococcus; GAS) [1–3]. To get more reproducible data it may be appropriate to investigate the different types of NSTI separately. NSTI caused by GAS seems to represent a specific

disease entity, characterized by frequent development of shock and high mortality. A substantial proportion of the patients have no predisposing comorbidity. The unique pathogenesis of streptococcal fasciitis is probably dependent on many virulence factors. Although the bacterial virulence profile appears to be critical for the development of NSTI and streptococcal toxic shock syndrome (STSS), the distribution of *emm* types, superantigens and other virulence factors varies in streptococcal isolates associated with severe invasive disease [4–6]. Only a few previous studies have investigated the characteristics of streptococcal NSTI in detail [7,8]. These studies have been confined to GAS infections, but recent studies have shown that *S. dysgalactiae* subsp. *equisimilis* of groups C and G (GCS and GGS) also have the ability to cause NSTI or STSS [9–13]. These two latter groups of streptococci share many virulence factors with GAS. Rarely, they even possess the group A carbohydrate antigen [14], which might lead to misidentification in the laboratory. To avoid this, additional tests for species identification can be used, although currently many laboratories use only serotype specificity on β-haemolytic streptococci and use the terms GAS and GCS/GGS. Streptococci within the *Streptococcus anginosus* group may also possess the group A, C or G antigens, but those can be easily discerned in the laboratory. In this study we describe incidence, clinical aspects and bacterial virulence gene profiles in NSTI caused by GAS and β-haemolytic GCS and GGS treated at Haukeland University Hospital, Norway during 2000–2009. The usefulness of the laboratory-based Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score for diagnosing streptococcal NSTI was also evaluated [15].

Patients and Methods

Study population, case definition and clinical parameters

Haukeland University Hospital (HUH) is a teaching hospital in Bergen, Norway. Patients admitted to the neighbouring Haraldsplass Deaconal Hospital are transferred to and treated at HUH when NSTI is suspected. These two hospitals have 1100 and 170 somatic beds, respectively. Incidence was calculated on the basis of cases from the catchment area of these two hospitals. The population in this area increased from *c.*347 000 to 387 000 inhabitants during the study period. HUH is also a tertiary hospital for a total of *c.*1 000 000 people. All cases of NSTI caused by GAS, GCS and GGS treated at HUH during 2000–2009 were retrospectively registered. Only the cases meeting surgical, clinical and microbiological criteria for streptococcal NSTI were included. NSTI was defined as necrotizing cellulitis or fasciitis as described previously [16]. Cases with surgical evidence of infectious myositis were also included, but cases of pyomyositis, defined as pus within individual muscle groups, were excluded [17]. Evaluation was based upon macroscopic findings during surgery, histological examination or post-mortem analyses. Removal of tissue was considered mandatory for the diagnosis of NSTI, apart from cases where surgical treatment was withheld because the chances of survival were considered to be very low. STSS was defined using criteria originally meant for GAS [18]. Streptococcal aetiology was defined by the isolation of GAS, GCS or GGS from blood or the infected soft tissue. Cases were not included if the streptococci were isolated from a mixed flora with more than two species. Relevant data were obtained from the medical records using a standard abstraction sheet. Age, gender, demographic information, comorbidities and laboratory data at the time of admission were recorded. Microbiological culture results, antibiotic and surgical treatment, per operative findings, relevant adjunctive therapy, and outcome were also recorded. Written informed consent from the patients was not needed, as judged by the Regional Committee of Medical and Health Research Ethics. The study was approved by the privacy ombudsman of HUH.

Bacterial identification

All GAS, GCS or GGS were β-haemolytic and formed large colonies on blood agar. Serogroup-specificity was determined using a rapid agglutination test (Oxoid, Basingstoke, Hampshire, UK). These methods of identification imply that rare cases of *S. dysgalactiae* subsp. *equisimilis* of serogroup A may have been misidentified as *S. pyogenes* (GAS).

Emm typing

Extraction of chromosomal DNA after mechanical lysis of the cell wall and *emm* typing were performed as previously described [19]. The following primers were used for initial *emm* amplification and sequencing in both directions: 5′-GGG-AAT-TCT-ATT-SGC-TTA-GAA-AAT-TAA-3′ (forward primer) and 5′-GCA-AGT-TCT-TCA-GCT-TGT-TT-3′ (reverse primer) [20]. Assignment of *emm* types was done using the CDC Streptococcus Laboratory *emm* type database (http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm).

Detection of streptococcal superantigen genes and *slaA*

A multiplex PCR with specific primer pairs for the 11 exotoxin genes *speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa* and *smeZ* was used as described in detail elsewhere [21]. To include allelic variations of the *smeZ* gene we also used simplex PCR with a previously described primer pair [22]. Simplex PCR amplifications of $speG_{dys}$, a *speG* orthologue found in *S. dysgalactiae* subsp. *equisimilis*, and the gene encoding strep-

tococcal phospholipase A2 (*SlaA*) were performed with primers described previously [9]. The $speG_{dys}$ primers amplified segments of equal size in both $speG_{dys}$ and *speG*, whereas the *speG* primers amplified only *speG*. Hence, all the GAS and GCS/GGS were screened for the presence of the 11 GAS superantigen genes (SAg) and *SlaA*, whereas only the GCS/GGS were subjected to PCR with the $speG_{dys}$ primers.

Statistical analysis

Data were analysed using SPSS PASW Statistics, version 20.0 (SPSS Inc, Chicago, IL, USA). Categorical data were analysed using Fisher's exact test. Non-parametric data (age) were analysed using the Mann–Whitney *U*-test. Because multiple comparisons were performed, both unadjusted and Holm–Bonferroni-corrected p values were calculated. A two-sided p-value $\leq$0.05 was considered statistically significant. To identify predictors of mortality, univariate analysis of several factors previously reported to be associated with mortality was first performed. Multivariate logistic regression analysis was performed choosing factors representing both predisposing conditions and acute disease severity and with low multicollinearity and low proportion of missing values.

Results

Seventy patients treated for NSTI caused by GAS, GCS or GGS in the period 2000–2009 were identified and included in the study. The mean annual incidence was 1.4 per 100 000 population. The annual incidence varied greatly, ranging from 0.29 to 2.84 per 100 000. Sixty-four of the infections were community-acquired, and no cases were epidemiologically linked. Sixty-one of the patients had NSTI caused by GAS, whereas GCS accounted for two and GGS for seven cases. *Staphylococcus aureus* was co-cultured with GAS in two samples and together with GGS in two samples. Median age at presentation was 55.5 years (range 4–87 years); and 55 and 72 years among GAS and GCS/GGS patients, respectively (p 0.11). Only one patient was below 16 years of age, and 47 of the patients were men. An overview of the clinical features, laboratory data, predisposing factors and outcome is shown in Table 1. In almost half of GAS patients both the muscle fascia (aponeurosis) and muscle tissue were affected, whereas only two of the patients with GCS/GGS infection had such signs of deep tissue involvement. A minority of the patients had only necrosis of subcutaneous tissue/superficial fascia without evidence of involvement of the muscle fascia or muscle. STSS was diagnosed more often in patients with muscle involvement than in the other patients (p 0.04). The lower limb was the site of infection in 35 of the patients. Most patients had positive tissue cultures even though most of them had received antibiotics before surgery. Only about half of the patients had an LRINEC laboratory score $\geq$6, which has been associated with a high probability of NSTI in a previous study on NSTI and cellulitis [15]. Significant comorbidity was present in less than half of the GAS patients, most commonly cardiovascular disease. Surgical treatment within 24 h of admittance was performed in 45 patients, and the median number of operative procedures performed per patient was three. The amputation rate was 8.6%. Clindamycin was included in the initial antibiotic treatment for 40 patients. Only one patient was treated with intravenous immunoglobulin, and none were treated with hyperbaric oxygen. The in-hospital case fatality rates (CFR) of GAS and

TABLE 1. Clinical characteristics and outcome

	GAS (*n* = 61)		GCS/GGS (*n* = 9)		All cases (*n* = 70)		
	No. with characteristic	Evaluable cases	No. with characteristic	Evaluable cases	No. with characteristic	Evaluable cases	p value[a]
Disease manifestations							
Subcutaneous involvement only[b,c]	5	59	3	7	8	66	0.033 (0.363)
Muscle involvement[b]	26	55	2	9	28	64	0.278
STSS	28	61	5	9	33	70	0.726
Laboratory data							
Blood culture positive	27	61	2	9	29	70	0.289
Tissue culture positive	44	60	8	9	52	69	0.435
LRINEC score $\geq$6[d]	29	58	2	9	31	67	0.161
Predisposing factors							
Age $\geq$65 years	15	61	5	9	20	70	0.107
Comorbidity	29	61	7	9	36	70	0.152
Outcome							
Amputations	6	61	0	9	6	70	1.000
Died in hospital	7	61	3	9	10	70	0.112
Died before 90 days	12	61	3	9	15	70	0.392

GAS, group A streptococcus; GCS, group C streptococcus; GGS, group G streptococcus; STSS, streptococcal toxic shock syndrome; LRINEC, Laboratory risk indicator for necrotizing fasciitis.
[a]For the difference between GAS and GCS/GGS. Fisher's exact test. In parenthesis: p value corrected for multiple comparisons (Holm–Bonferroni method).
[b]Based on macroscopic findings during surgery.
[c]Muscle fascia (aponeurosis) and muscle tissue not involved.
[d]Based on values for C-reactive protein, leucocytes, haemoglobin, creatinine, glucose and sodium [15].

TABLE 2. Predictors of in-hospital mortality

	Survivals (*n* = 60)		Deaths (*n* = 10)			
	No. with characteristic	Evaluable cases	No. with characteristic	Evaluable cases	OR (95% CI)[a]	p value[b]
STSS	24	60	9	10	13.50 (1.61–113.55)	0.005 (0.045)
Muscle involvement[c]	20	55	8	9	14.00 (1.63–120.22)	0.008 (0.056)
Bacteraemia	23	60	6	10	2.41 (0.61–9.48)	0.299
Surgery >24 h[d]	22	60	3	10	0.74 (0.17–3.16)	1.000
Surgical revisions <3[e]	8	60	6	10	9.75 (2.25–42.32)	0.003 (0.033)
Not clindamycin[d]	27	60	3	10	0.52 (0.12–2.22)	0.498
Age ≥65 years	13	60	7	10	8.44 (1.91–37.26)	0.004 (0.040)
Comorbidity	30	60	6	10	1.50 (0.384–5.860)	0.736
Heart disease	6	60	5	10	9.00 (2.01–40.30)	0.006 (0.048)
LRINEC score ≥6[f]	26	58	5	9	1.54 (0.37–6.32)	0.723
emm type 1/3/5/12/18/87/89[g]	19	36	4	6	1.79 (0.29–11.04)	0.673

STSS, streptococcal toxic shock syndrome; LRINEC, Laboratory risk indicator for necrotizing fasciitis.
[a]ORs and 95% CIs were estimated using univariate logistic regression analysis.
[b]Fisher's exact test. In parentheses: p values corrected for multiple comparisons (Holm–Bonferroni method).
[c]Based on macroscopic findings during surgery.
[d]From admittance.
[e]Fewer than three surgical revisions before discharge/death.
[f]Based on values for C-reactive protein, leucocytes, haemoglobin, creatinine, glucose and sodium [15].
[g]*emm* types in GAS cases associated with case fatality rate >30% in necrotizing fasciitis [23].

TABLE 3. Virulence genes of 42 group A streptococcus isolates

emm type[a]	No.	speA	speC	speG	speH	speI	speJ	speK	speL	speM	ssa	smeZ	SlaA
*emm*1.0	13[b]	+	–	+	–	–	+	–	–	–	–	+	–
*emm*3.1	5	+	–	+	–	–	–	+	–	–	+	+	+
*emm*4.0	4	+(1)/–	+/–(1)	–	–	–	–	–	–	–	+	+	–
*emm*9.0	2	–	–	+	–	–	–	+/–	–	–	+	+	–
*emm*28.0	3	+(1)/–	+	+	–	+	–	+(2)/–	–	–	–	+	+(2)/–
*emm*82.0	2	–	+	+	+	+	–	–	–	–	–	+	–
*emm*89.0	2	–	+/–	+	–	–	+/–	+/–	–	–	–	+	+/–

Nineteen group A streptococcus isolates were not available for analysis.
[a]Other *emm* types (*n* = 1): *emm*2.0: speC+speG, *emm*6.0: speA+ speC+speG +speK+smeZ+SlaA, *emm*12.0: speC+speG +speH+speI+smeZ, *emm*43.4: speG+speK+smeZ+SlaA, *emm*49.0: speC+ speG+speH+speI+speL, *emm*75.0: speC+speG +speL+speK+speM+smeZ+slaA, *emm*77.0: speC+smeZ, *emm*78.3: speG+smeZ, *emm*87.0: speG+speH+speI+speJ+smeZ, *emm*96.0: speC+speG, *emm*102b: speC+speG +speJ+smeZ.
[b]Including one isolate with subtype 1.22.

GGS disease were 11% and 33%, respectively, whereas the CFR after 3 months were 20% and 33%, respectively.

The impact of various factors on in-hospital mortality is presented in Table 2. Premorbid factors (age ≥65 years and comorbid heart disease) as well as disease manifestations (STSS, muscle involvement) and treatment factors (fewer than three surgical debridements) were associated with mortality in the univariate analysis. When corrected for multiple comparisons, muscle involvement was not a significant factor. The association between mortality and few debridements could be explained by the fact that in five of the six patients with fewer than three debridements who died, surgical treatment was withheld because of a moribund state. In a logistic regression analysis age, STSS and comorbidity were chosen as predictor variables. Independent predictors of mortality in this model were increased age (OR per year 1.095, 95% CI 1.023–1.172; p 0.009) and STSS (OR 9.596; 95% CI 1.016–90.640; p 0.048).

Forty-two GAS isolates were available for molecular analysis. The *emm* type distribution in relation to SAg gene profile of the GAS isolates is shown in Table 3. Thirteen isolates possessed *emm*1. All isolates except those possessing *emm*4.0 and *emm*77.0 contained *speG*, and all but three isolates, belonging to *emm*2, *emm*49 and *emm*96 contained *smeZ*. All the 13 isolates of *emm*1 showed identical SAg profiles and contained the phage-mediated superantigen *speA* and the chromosomally encoded *speG*, *speJ* and *smeZ*. For other *emm* types found in more than one case, the isolates sharing the same *emm* type also had the same virulence gene profile in all but four isolates. The four available GCS/GGS isolates belonged to four different *emm* types (*stC*74a.0, *stG*10.0, *stG*2574.0, *stG*6.3). The $speG_{dys}$ gene was found in isolates of the former three of these *emm* types. GAS SAg or *SlaA* genes were not identified in GCS/GGS.

There was no significant correlation between fatal outcome and specific virulence gene profiles, although three of the six genotyped GAS isolates associated with fatal outcome had *emm* types previously reported to give a CFR above 30% when associated with NF [23].

Discussion

The present study is among the largest series of streptococcal NSTI reported to date. Several large series of NSTI have been

published during the past few years, but none of these presented more than a few clinical parameters in relation to bacterial aetiology [24–27]. Detailed and stringent clinical and microbiological information from large series on streptococcal NSTI is lacking, apart from prospective studies from Ontario, Canada performed in the 1990s [7,8]. Compared with the incidence of GAS NF in prospective studies from Canada (0.08–0.49/100 000/year) and Europe (0.22–0.6/100.000/year) a relatively high frequency of streptococcal NSTI was observed in our study, and GCS/GGS infections contribute to the disease burden [8,23,28]. Differences in reported incidence rates may also be explained by the use of imprecise inclusion criteria. In studies on NF, cases with engagement of the muscle fascia are probably often the only ones included. Excluding cases without such involvement might be inappropriate, because streptococcal disease with rapid spread along the subcutaneous tissue planes probably represents the same disease as the slightly more deeply situated infections. To improve comparability of studies, it may be more feasible to use the term NSTI rather than NF, as suggested by several authors, implying that cases are included irrespective of soft tissue level [2,3,29,30]. Almost half of our patients had involvement of the muscle fascia and muscle tissue. The incidence in our study is therefore high even when comparing only definite NF cases.

The high proportion of patients with intraoperative signs of muscle tissue involvement shows that streptococcal infections not only spread along tissue planes; they often disrespect borders between tissue compartments. GAS-infected patients had muscle tissue involvement more often than GCS/GGS-infected patients. However, the difference was not statistically significant, and the number of GCS/GGS cases was low.

During the study period, <5% of all NSTI (type I and type II infections) at our hospital were culture negative (data not shown). This leads us to believe that almost all streptococcal cases treated in our institution were included in this study. High bacterial load and reduced bioavailability of antibiotics are known to reduce the effect of antibiotics in NSTI. These observations are supported by the high number of positive tissue cultures among the included patients, although most patients received antibiotic treatment before surgical intervention.

The LRINEC score was an insensitive tool for the diagnosis of streptococcal NSTI in our patients. A possible explanation might be the high awareness of NSTI in our region, contributing to early hospitalization and thus a low LRINEC score at admission. The usefulness of this score has been questioned also in several other recent studies [2,3]. Early recognition of typical symptoms and signs that requires surgical exploration therefore remains the major tool in the diagnosis of streptococcal NSTI [3]. High-quality education and training of the physicians involved is therefore essential.

The significant proportion of streptococcal NSTI caused by GCS/GGS in our study confirms the ability of these streptococci to cause severe soft tissue infections. In accordance with other studies of invasive GCS and GGS disease, our GCS/GGS-infected patients tended to be older and more often had underlying disease than GAS patients [9–12]. Among the 61 GAS cases 27 neither had comorbidity nor age ≥65 years, supporting the impression that GAS are highly virulent bacteria able to cause particularly severe soft tissue infections even in patients without predisposing conditions [7].

Although the CFR of GAS infections in the present study was relatively low, several factors were found to be associated with mortality. A high CFR in those with muscle tissue involvement found here is consistent with the high mortality previously reported in streptococcal myositis, a particularly severe form of NSTI [31]. Our findings differ from observations reported in a study of NSTI where the presence of myonecrosis did not influence mortality. This is possibly explained by a low proportion of streptococcal disease [29]. We found that STSS and age are independent predictors of mortality. These results are consistent with those from other studies [7,8,25,26]. Even though early surgery or clindamycin treatment was not significantly associated with favourable outcome in this material, these factors were protective in other series [1].

Certain *emm* types, like *emm*1, *emm*3, *emm*4 and *emm*28, dominated our GAS samples. Particularly *emm1* and *emm3* have previously been linked to NSTI, STSS and mortality, probably indicating that isolates within certain *emm* types are genetically fit to produce more severe disease manifestations [4,23]. Nevertheless, the overall *emm* type diversity in our material was substantial. This, along with a lack of correlation between *emm* type/superantigen gene profile and fatal outcome, indicates that host susceptibility is a key factor in the development and course of streptococcal NF. The low content of superantigen genes detected in GCS/GGS probably reflects the fact that these virulence factors are not a prerequisite for the development of NSTI caused by these bacteria [9,10,12].

The major limitations of this study arise from the retrospective inclusion of data. Such a design makes it difficult to perform a detailed and standardized characterization of all the patients, including preoperative clinical findings, macroscopic findings upon surgery and comprehensive laboratory analyses.

In conclusion, we report a relatively high incidence of streptococcal NSTI in our region of western Norway. GCS and GGS infections contributed to the disease burden, but they differ from GAS cases in frequency and predisposing factors.

The overall mortality rates were relatively low, probably related to well-implemented routines of early surgery, appropriate antibiotic therapy and adequate supportive measures.

Acknowledgements

We thank M. Gjerde Tellevik (Centre for Tropical and Imported Infectious Diseases, Haukeland University Hospital) for excellent technical support. Preliminary results for some of the patients were presented at the 18th Lancefield International Symposium in Italy, September 2011 (abstract book p.41). The study was supported by the Department of Clinical Science, University of Bergen. The results form part of the clinical background material for the EU FP7-funded INFECT study (www.fp7infect.eu).

Transparency Declaration

The authors declare that there are no potential conflicts of interests.

References

1. Sarani B, Strong M, Pascual J, Schwab CW. Necrotizing fasciitis: current concepts and review of the literature. *J Am Coll Surg* 2009; 208: 279–288.
2. Phan HH, Cocanour CS. Necrotizing soft tissue infections in the intensive care unit. *Crit Care Med* 2010; 38(suppl): 460–468.
3. Ustin JS, Malangoni MA. Necrotizing soft-tissue infections. *Crit Care Med* 2011; 39: 2156–2162.
4. Olsen RJ, Musser JM. Molecular pathogenesis of necrotizing fasciitis. *Annu Rev Pathol* 2010; 5: 1–31.
5. Lintges M, Van der Linden M, Hilgers RD *et al.* Superantigen genes are more important than the *emm* type for the invasiveness of group A Streptococcus infection. *J Infect Dis* 2010; 202: 20–28.
6. Lynskey NN, Lawrenson RA, Sriskandan S. New understandings in *Streptococcus pyogenes*. *Curr Opin Infect Dis* 2011; 24: 196–202.
7. Kaul R, McGeer A, Low DE, Green K, Schwartz B, Simor AE. Population-based surveillance for group A streptococcal necrotizing fascitis: clinical features, prognostic indicators, and microbiologic analysis of seventy-seven cases. *Am J Med* 1997; 103: 18–24.
8. Sharkawy A, Low DE, Saginur R *et al.* Severe group A streptococcal soft-tissue infections in Ontario: 1992–1996. *Clin Infect Dis* 2002; 34: 454–460.
9. Ikebe T, Murayama S, Saitoh K *et al.* Surveillance of severe invasive group-G streptococcal infections and molecular typing of the isolates in Japan. *Epidemiol Infect* 2004; 132: 145–149.
10. Brandt CM, Spellerberg B. Human infections due to *Streptococcus dysgalactiae* subspecies *equisimilis*. *Clin Infect Dis* 2009; 49: 766–772.
11. Takahashi T, Sunaoshi K, Sunakawa K, Fujishima S, Watanabe H, Ubukata K. Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections. *Clin Microbiol Infect* 2010; 16: 1097–1103.
12. Ikebe T, Oguro Y, Ogata K *et al.* Surveillance of severe invasive group G streptococcal infections in Japan during 2002–2008. *Jpn J Infect Dis* 2010; 63: 372–375.
13. Kittang BR, Langeland N, Skrede S, Mylvaganam H. Two unusual cases of severe soft tissue infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*. *J Clin Microbiol* 2010; 48: 1484–1487.
14. Jensen A, Kilian M. Delineation of *Streptococcus dysgalactiae*, its subspecies, and its clinical and phylogenetic relationship to *Streptococcus pyogenes*. *J Clin Microbiol* 2012; 50: 113–126.
15. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. *Crit Care Med* 2004; 32: 1535–1541.
16. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 2005; 33: 1538–1548.
17. Stevens DL, Bisno AL, Chambers HF *et al.* Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 2005; 41: 1373–1406.
18. Breiman RF, Davis JP, Facklam RR, Gray BM, Hoge CW, Kaplan EL. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. *JAMA* 1993; 269: 390–391.
19. Kittang BR, Langeland N, Mylvaganam H. Distribution of *emm* types and subtypes among noninvasive group A, C and G streptococcal isolates in western Norway. *APMIS* 2008; 116: 457–464.
20. Musser JM, Kapur V, Szeto J, Pan X, Swanson DS, Martin DR. Genetic diversity and relationships among serotype M1 strains of *Streptococcus pyogenes*. *Dev Biol Stand* 1995; 85: 209–213.
21. Lintges M, Arlt S, Uciechowski P *et al.* A new closed-tube multiplex real-time PCR to detect eleven superantigens of *Streptococcus pyogenes* identifies a strain without superantigen activity. *Int J Med Microbiol* 2007; 297: 471–478.
22. Darenberg J, Luca-Harari B, Jasir A *et al.* Molecular and clinical characteristics of invasive group A streptococcal infection in Sweden. *Clin Infect Dis* 2007; 45: 450–458.
23. Luca-Harari B, Darenberg J, Neal S *et al.* Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2009; 47: 1155–1165.
24. Anaya DA, Bulger EM, Kwon YS, Kao LS, Evans H, Nathens AB. Predicting death in necrotizing soft tissue infections: a clinical score. *Surg Infect (Larchmt)* 2009; 10: 517–522.
25. Huang KF, Hung MH, Lin YS *et al.* Independent predictors of mortality for necrotizing fasciitis: a retrospective analysis in a single institution. *J Trauma* 2011; 71: 467–473.
26. Chen IC, Li WC, Hong YC, Shie SS, Fann WC, Hsiao CT. The microbiological profile and presence of bloodstream infection influence mortality rates in necrotizing fasciitis. *Crit Care* 2011; 15: R152.
27. Das DK, Baker MG, Venugopal K. Risk factors, microbiological findings and outcomes of necrotizing fasciitis in New Zealand: a retrospective chart review. *BMC Infect Dis* 2012; 12: 348.
28. Plainvert C, Doloy A, Loubinoux J *et al.* Invasive group A streptococcal infections in adults, France (2006–2010). *Clin Microbiol Infect* 2012; 18: 702–710.
29. Elliott DC, Kufera JA, Myers RA. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. *Ann Surg* 1996; 224: 672–683.
30. Anaya DA, McMahon K, Nathens AB, Sullivan SR, Foy H, Bulger E. Predictors of mortality and limb loss in necrotizing soft tissue infections. *Arch Surg* 2005; 140: 151–157.
31. Crum-Cianflone NF. Infection and musculoskeletal conditions: infectious myositis. *Best Pract Res Clin Rheumatol* 2006; 20: 1083–1097.